Aflatoxin Inactivation in Corn by Aqua Ammonia

O. L. Brekke, A. J. Peplinski, E. B. Lancaster

ABSTRACT

AFLATOXIN, a potent toxin sometimes found in corn, can cause serious economic problems for the animal-production industry. In laboratory tests, aqueous ammonia effectively reduced the acute toxicity of the toxin in naturally contaminated corn as determined by chemical analysis and verified by biological assays. Results are presented on the effects of temperature, ammonia and moisture levels in corn, and reaction time on the response of several corn lots varying in initial aflatoxin content. Drying to reduce the ammonia odor and taste is necessary for animal acceptance of the treated corn.

INTRODUCTION

Aflatoxin, a harmful secondary metabolite, can be produced on corn by widely distributed strains of two common molds, *Aspergillus flavus* and *A. parasiticus.* The toxin's dietary effect upon poultry can result in poor growth, increased mortality, poor feed conversion, and increased condemnations (Smith and Hamilton, 1970). A number of other animal species are also subject to aflatoxicosis. Aflatoxin has been known to act as a potent toxin, a carcinogen, a teratogen, and a mutagen (Ciegler, 1975). Several excellent reviews covering many aspects of the aflatoxin problem have been published (Goldblatt, 1969a; Lillehoj et al., 1970; Detroy et al., 1971; Campbell and Stoloff, 1974; Ciegler, 1975). Action by the Food and Drug Administration (FDA) in condemning contaminated foodstuffs, and some agricultural commodities and feedstuffs emphasizes this agency's concern about the aflatoxin problem (Schmidt, 1974; Anonymous, 1971; Rodricks, 1975; and Stoloff, 1972).

Conditions that lead to mycotoxin contamination in cereals, foods, and feeds have been reported by Hesseltine (1974, 1976). Aflatoxin may be more prevalent in corn grown in southern parts of the United States than that grown in the so-called corn belt (Shotwell et al., 1973). Also, possible contamination of corn in the field is becoming increasingly evident (Anderson et al., 1975; Lillehoj et al., 1975a, 1975b, 1976a, 1976b; Zuber et al., 1976), *A. flavus* and *A. parasiticus* normally are considered as active primarily in stored grain.

Attempts at Removal or Inactivation of Aflatoxin in Various Agricultural Commodities

Various ways for removal or inactivation of aflatoxin based on physical, chemical, or biological means have been and are being studied. While most physical separation methods are relatively inexpensive, their success usually is limited to commodities wherein aflatoxin contamination is found in a relatively small proportion of the seed whose physical characteristics have been changed sufficiently to make the separation possible. A combination of microscopic examination and physical separation methods is used during the processing of peanuts into food products meeting the current aflatoxin tolerance level set by the FDA (Kensler and Natoli, 1969). However, dry- and wet-cleaning methods did not satisfactorily remove aflatoxin from naturally contaminated corn unless the toxin was in a fraction readily removable by a simple sieving operation (Brekke et al., 1975a). Neither the wet-milling process (Yahl et al., 1971) nor the dry-milling process (Brekke et al., 1975b) removed or inactivated the aflatoxin in corn. While aflatoxins are relatively stable to heat, roasting of peanuts lowered the aflatoxin content (Lee et al., 1969) as did the cooking of oilseed meals (Dollear, 1969) but not to the degree always needed to meet FDA's tolerance level. A high degree of removal has been attained by solvent extraction of oilseed meals (Vorster, 1966; Prevot, 1974) but the process has economic and other limitations.

A number of chemicals have been investigated for their ability to either destroy, transform, or inactivate aflatoxin (Dollear, 1969; Mann et al., 1970; Mann et al., 1971; and Goldblatt, 1971). Researchers at the Southern and the Western Regional Research Centers found ammonia to be one of the more effective reagents for practical treatment of contaminated cottonseed and peanut meals (Masri et al., 1969).

The reaction between aflatoxin B_1, ammonium hydroxide, and corn flour has been studied by Beckwith et al. (1975, 1976). Others have reported on the identi-

Article was submitted for publication in March 1977; reviewed and approved for publication by the Electric Power and Processing Division of ASAE in June 1977. Presented as ASAE Paper No. 75-3507.

The authors are: O. L. BREKKE, Research Leader (retired), A. J. PEPLINSKI, Chemical Engineer, and E. B. LANCASTER, Chemical Engineer (retired), Northern Regional Research Center, USDA-ARS, Peoria, IL.

Acknowledgments: The authors thank Paul Brooks, James Mauer and G. W. Nofsinger for technical assistance; O. L. Shotwell, E. Vandegraft and coworkers for the aflatoxin assays: L. T. Black and coworkers for other chemical assays; Alex Ciegler for arranging for biological assays; W. F. Kowlek (Biometrican, North Central Region, USDA, stationed at Northern Center) for statistical analyses; Peoria office of the Grain Division, AMS, USDA for corn grading tests; and suggestions and advice by E. L. Griffin, Jr., Alex Ciegler and E. B. Bagley.

We also thank the following for permission to quote from results of the biological tests: C. C. Calvert, ARS-USDA for laying hen tests; James Dizikes, Dairy Division Lab., AMS-USDA for taste panel tests on eggs; P. B. Hamilton, North Carolina State University, Raleigh, for broiler chick tests; A. H. Jensen, University of Illinois, Urbana, for swine tests; and R. O. Sinnhuber, Oregon State University, Corvallis, for trout tests.

This article is reprinted from the TRANSACTIONS of the ASAE (Vol. 20, No. 6, pp. 1160, 1161, 1162, 1163, 1164, 1165, 1166, 1167, 1168, 1977)
Published by the American Society of Agricultural Engineers, St. Joseph, Michigan

TABLE 1. SELECTED CHARACTERISTICS OF NATURALLY CONTAMINATED CORN.

Lot no.	US grade	BCFM* percent	DKT† percent	Aflatoxin, µg/kg‡ Total	B_1	B_2	G_1	G_2
7	1	1	2	32	30 (16-45)	2 (1-3)	NA§	NA
104	1	+‖	+	240	220 (140-260)	16 (10-21)	ND**	ND
102	3	0	5	220	190	23	4	ND
103	4	4	8	160	140	12	3	1
105	5	+	15	550	510 (320-610)	42 (13-50)	NA	NA
2	SG††	+	15	34	31 (18-50)	3 (2-7)	NA	NA
107	SG	9	5	130	120	8	ND	ND
108	SG	1	8	360	310 (220-520)	36 (24-26)	12 (5-21)	2 (2-3)
125	SG	2	14	1200	1000 (840-1390)	90 (70-120)	80 (ND-240)	2 (ND-23)
113	SG	1	16	1300	1200 (1150-1220)	120 (118-122)	NA	NA

*Broken corn and foreign material.
†Damaged kernels, total.
‡Average values with range given in parentheses if more than one sample was analyzed.
§Not analyzed.
‖Less than 1 percent.
**None detected.
††Sample grade.

fication and characterization of compounds formed by the reaction between aflatoxin and ammonium hydroxide under various conditions (Lee et al., 1974; Cuculla et al., 1975; Stanley et al., 1975; and Vesonder et al., 1975).

After a review of the literature and a few preliminary experiments with various chemicals, we concluded that ammonia could be an effective chemical for reducing the toxicity of aflatoxin in corn. We undertook adaptation of the Southern Regional Research Center process (Gardner et al., 1971; McKinney et al., 1973) to corn with the ammoniation being conducted under atmospheric rather than elevated pressures. Many of our laboratory-scale experiments were made by mixing corn with an aqueous solution of ammonia. For the present report, we have studied the effect of the following reaction variables: percent ammonia added, reaction temperature, reaction time, moisture content of the corn at time of ammoniation, and variation in response among several lots of corn varying in their initial aflatoxin content. Some data on drying and animal acceptance of the ammoniated corn are also included.

MATERIALS AND METHODS

Ammonia

Generally, the aqueous ammonia solutions were of chemical grade; some of fertilizer grade were used in preparation of material for animal feeding tests. Concentration of ammonia in the solutions varied between 18 and 28 percent by weight.

Corn

Both yellow and white corn from several geographic locations were used. Selected characteristics of the individual lots are given in Table 1. All lots were naturally contaminated and their aflatoxin B_1 content varied between 30 and 1200 µg/kg. Because of the heterogeneous nature of the contamination, a fact well recognized by those working in this field, the aflatoxin content of practically every lot varied considerably even after blending in the usually acceptable manner. Whenever several samples from a given lot were analyzed, the range in aflatoxin content as well as the average value is given. In the later discussion of specific experiments, aflatoxin content of the sublot used is given whenever possible.

Ammoniation Procedure

For a number of experiments, the corn, usually 3.6 kg (8 lb), was placed in a double plastic (2-mil polyethylene) bag, aqua ammonia and any necessary water added, the bag tied shut, contents mixed by hand manipulation for a few minutes, and the sample then stored at a specified temperature. For experiments at elevated temperatures the corn was preheated, and the tied bags were placed in metal cans having tight fitting slip covers which were further sealed with tape. Treated samples were held for specified time periods at -18, 1, 25, 38, 40, 49, or 60 °C or at room temperature. Then the entire sample was ground to -20 mesh (0.84 mm sieve openings), blended, and about 150 g neutralized for aflatoxin analysis.

FDA's action guideline or tolerance level for total aflatoxins in foods and feeds is 20 µg/kg (B_1, B_2, G_1, and G_2) which has stood since 1969 (Rodricks, 1975). Recently, FDA published a proposal to lower the action level for total aflatoxin content of shelled peanuts and peanut products from 20 to 15 ppb (Schmidt, 1974). However, as of this writing, the proposal has not been put into effect. Because aflatoxin B_1 often constitutes 75 percent or more of the total aflatoxin content of corn (Table 1), we arbitrarily set 15 µg/kg of aflatoxin B_1 as the upper residual limit and a nondetectable level (ND) as the ultimate goal in ammoniated corn. Usage of ammoniated corn presumably will be limited to animal feeding.

Ammonia applied to the corn is expressed as weight percent (0.3 to 4.0), based on dry matter content of the corn. Moisture content of the treated corn is expressed as weight percent, wet basis (12 to 25 percent range) with added ammonia disregarded.

Detoxification of Contaminated Corn for Feeding Trials

Feeding tests were made with ducklings, broiler chicks, and trout to establish validity of the chemical assays used in the laboratory detoxification experiments. The corn was treated by addition of sufficient aqua NH^3 to 5- to 250-kg quantities of corn to provide 1.5 percent NH_3. Moisture content of the corn was adjusted to 17.5 by addition of tap water.

For the trout test, the corn (57 kg) was heated to 49 °C in a 208-L (55-gal) closed drum and held at that temperature for 13 days. At the end of the reaction period, the corn was ground with 95 percent passing through a sieve having 0.42-mm openings, then heated (42-48 °C), and aerated in a jacketed, steam-heated ribbon blender to remove the ammonia odor.

A 250-kg batch of corn for the broiler chick feeding test was ammoniated in a 0.56 m^3 (20 ft^3) double cone mixer heated electrically with manual temperature control. The corn was heated only during the working hours of 3 nonconsecutive days and allowed to cool in between times. The temperature varied between 25 and 47 °C and after a 19-day reaction period, the corn was ground and deodorized as above. Also, noncontaminated corn for a second broiler chick test was divided into several sublots which were ammoniated in closed 208 L drums at various temperatures, times, and ammonia levels, then ground and deodorized as for the trout test.

For the duckling test, 4.5 kg of the ammoniated corn was held for 7 mo at 18-38 °C, then ground and aerated with room air (20-26 °C) for 16 hr in a recirculating air dryer to remove most of the ammonia odor. A preliminary test had demonstrated the need for deodorizing the corn because ducklings refused to eat corn which had not been deodorized.

Drying Procedure

We made two drying tests with 7.2 kg of ammoniated whole corn placed in a layer about 2.5 cm deep in a perforated tray. Air heated to 52 or 93 °C was passed downflow through the corn in a laboratory-size, variable-flow recirculation dryer. Periodically, the tray and contents were removed and weighed and the contents mixed, sampled, and analyzed for residual amounts of total volatiles and water-extracted ammonia (WE-NH_3).

Neutralization of Ammoniated Samples for Aflatoxin Assay

At the end of the specified reaction period, the sample was ground in a hammer mill and blended (these steps took about 20 min), then neutralized as follows. Approximately 150 g of ammoniated, ground corn was mixed with 700 ml distilled water, and neutralized immediately under continuous agitation with standardized sulfuric acid (ca 1.7 N) to a final pH reading of 5.7 after 20 min. The readings were made with a pH meter and pH was not allowed to drop below 5.0 at any time during acid addition. The mixture was filtered through a No. 1 Whatman filter paper under about 660 mm Hg vacuum and the filter cake dried (often overnight) in a forced draft oven, operating at 25, 49, or 60 °C with the latter preferred. The cake was ground to a fine powder with a mortar and pestle and blended before assay. Solids recovery was about 90 percent. To ascertain that aflatoxin content of the sample was not altered by this procedure, duplicate samples were analyzed on two occasions. One sample was neutralized by the above procedure and the other sample was neutralized while in direct contact with the extraction solvent used in the analytical determination. Aflatoxin determinations on duplicate samples agreed within the limits of analytical error. Also, the filtrates from two samples were analyzed and found to contain no aflatoxin.

TABLE 2. EFFECT OF AMMONIA AND MOISTURE LEVELS IN TREATED CORN ON RESIDUAL AFLATOXIN B_1 CONTENT FOR REACTIONS AT 10, 25 AND 40 °C.

NH_3 added, percent of dry matter	Corn moisture, percent w.b. 12.5	15.0	17.5	20.0	22.5	25.0
	Residual aflatoxin B_1, µg/kg*					
	42 days at 10 °C					
0.5	400	180	90	50	34	34
1.0	250	80	40	13	11	17
1.5	260	40	12	17	13	27
2.0	260	16	21	14	19	27
3.0	—	60	23	16	12	15
	8 days at 25 °C					
0.5	200	100	43	45		
1.0	49	35	30	7		
1.5	36	24	15	12		
2.0	—	17	10	26		
	2 days at 40 °C†					
0.5	110	44	28	23		
1.0	28	9.0	10.3	7.7		
1.5	10.0	6.3	6.0	8.8		
2.0	—	7.6	10.1	12.0		

*Initial aflatoxin B_1 content was 1000 µg/kg (lot 125).
+Average of two sets of values.

Analytical Methods

Quantitative aflatoxin determinations were made on 50-g portions of the ground corn before and after ammoniation by the method recommended for corn (AOAC, 1975). Reported values are based on single determinations unless otherwise noted. For a single determination on samples of ground, unammoniated corn, Shotwell et al. (1975a) have reported the relative standard deviation (RSD) as 37 percent, which decreases to 25 percent for two determinations and to 20 percent for three determinations. Sensitivity of the method is 1-3 µg/kg (Shotwell et al., 1975b). Aflatoxin values above 1000 µg/kg have been rounded off at the nearest multiple of 100, between 1000 and 50 µg/kg to the nearest multiple of 10, and below 50 µg/kg to the nearest whole integer except for the part in Table 2 where two independent sets of experiments were made at 40 °C and the values averaged.

Ammoniation conditions that reduced aflatoxin B_1 to low or nondetectable levels generally reduced the other aflatoxins by essentially the same or greater degree. With aflatoxin B_1 being the type most pre-

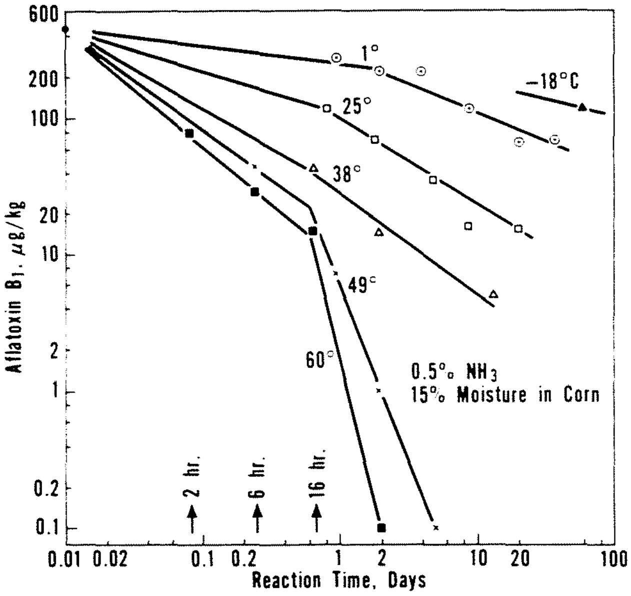

FIG. 1 Effect of temperature on rate of aflatoxin B_1 inactivation. After addition of 0.5 percent ammonia and adjustment of corn moisture to 15 percent [w.b.], samples [3.6 kg] were held for varying lengths of time at temperatures indicated.

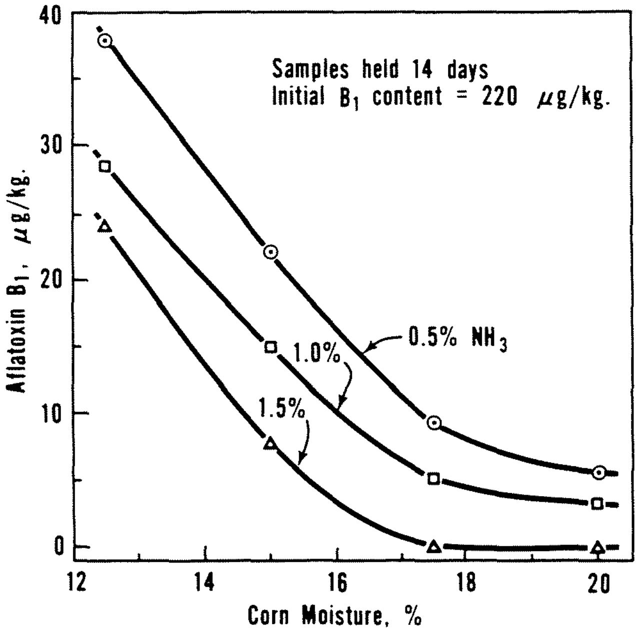

FIG. 2 Effect of ammonia level and corn moisture level on extent of aflatoxin B_1 inactivation. Samples were held for 14 days at 25 °C. Initial aflatoxin B_1 content of corn was 220 µg/kg [lot 108].

valent in the corn lots used and also the most toxic, emphasis was placed upon inactivation of B_1. Analytical difficulties precluded the determination of B_2, G_1, and G_2 aflatoxins in the ammoniated samples.

Methods used to determine total nitrogen content of the corn and the various forms of ammonia (i.e., ammoniacal nitrogen and water extracted) in the corn have been reported previously by Lancaster et al. (1974) and Uhl et al. (1971). Water-extracted ammonia (WE-NH^3) is the same as Lancaster et al.'s "free" ammonia and for the determination the sample size was increased from the 10 g recommended by Lancaster et al. to 20 g. Moisture content of the corn was taken as the loss in weight when ca. 200 g of whole-kernel corn was heated in a 103 °C forced draft oven for 72 hr (Anonymous, 1959) or when ca. 10 g of ground corn was heated for 30 min in a Brabender moisture oven set at 130 °C. If ammonia was present, the weight loss is reported as moisture and volatile matter (M&VM).

Corn samples were graded by the USDA standard procedure (Anonymous, 1970).

RESULTS AND DISCUSSION

Aflatoxin Inactivation: Effect of Temperature

Reaction temperature had a strong effect on rate and extent of aflatoxin B_1 inactivation as shown in Fig. 1. With 0.5 percent ammonia added, moisture content of the treated corn adjusted to 15 percent, and the reaction conducted at 25 °C, 20 days were required to reduce the aflatoxin B_1 content from 450 µg/kg (lot 105) to 15 µg/kg, the maximum acceptable residual level as arbitrarily set. With temperatures above 25 °C, appreciably less time was required to reach 15 µg/kg; after 5 days at 49 °C or 2 days at 60 °C, aflatoxin was nondetectable (ND, plotted as 0.1 µg/kg in Fig. 1).

Storage of an ammoniated sample first at a subfreezing temperature and then at an elevated temperature was not as effective in detoxifying the corn as if the sample had been held only at the elevated temperature. A sample from lot 108 (450 µg B_1/kg) treated with 0.5 percent ammonia at 15 percent moisture content, held first at -18 C for 69 days and then for 3 days at 49 °C, had a residual aflatoxin B_1 content of 11 µg/kg. The data in Fig. 1 indicate that if the sample had been stored directly at 49 °C for 3 days, its B_1 content would have lowered to 1 µg/kg. While low temperatures will reduce the aqua ammonia's volatility and thus reduce potential material handling losses in commercial applications, temperature of the ammoniated corn should be adjusted to 20 °C or higher for adequate detoxification within a normally acceptable time span.

Aflatoxin Inactivation: Effect of Ammonia and Moisture Levels

Fig. 2 shows the pronounced effect of corn moisture and ammonia level when samples were held for 14 days at 25 °C. With 0.5 percent ammonia, the aflatoxin B_1 level was reduced from 220 µg/kg (lot 108) to 15 µg/kg or less only at moisture contents of 16 percent and above. Increasing the ammonia level to 1.0 percent inactivated more of the aflatoxin and with 1.5 percent, no aflatoxin was detected at moisture levels of 17.5 and 20 percent.

Analogous results were obtained when corn (lot 125) containing 100 µg/kg of aflatoxin B_1 was ammoniated at 10, 25, or 40 °C (Table 2). At 10 °C, little or no additional detoxification occurred when the ammonia level was increased from 2 to 3 percent or when the corn moisture was increased above 20 percent. For the series of samples held 2 days at 40 °C and treated with 1, 1.5, or 2 percent ammonia at corn moistures of 15 to 20 percent, residual aflatoxin B_1 content appeared to reach a plateau at an average value of 8.6 µg/kg. Because of its higher aflatoxin content, lot 125 was more difficult to detoxify than was lot 108.

Data from the 8-day reaction at 25 °C indicate that 1.5 or 2.0 percent ammonia and about 17.5 percent moisture were required to reach a residual B_1 level of

TABLE 3. RESPONSE OF VARIOUS CONTAMINATED CORN LOTS TO A UNIFORM AMMONIATION TREATMENT*.

Lot no.	Moisture in ammoniated corn, percent, w.b.	Aflatoxin B_1, $\mu g/kg$ Initial	Residual
7C	15	30	ND†
2C	14	31	ND
107	15	120	ND
102	15	140	ND
103	14	160	1‡
108	14	440	9
105	15	450	9
125	15	1000	22§

*Samples held 14 days at 25 °C after addition of aqua ammonia at rate of 1 g NH_3 per 100 g dry matter in corn.
†None detected.
‡Extrapolated from 2 $\mu g/kg$ at 8 days.
§Extrapolated from 35 $\mu g/kg$ at 8 days.

TABLE 4. EFFECT OF AMMONIATION CONDITIONS UPON AFLATOXIN INACTIVATION—CORN LOT 104.

NH_3 added, percent of dry matter	Moisture in ammoniated corn, percent, w.b.	Reaction time, days	Residual aflatoxin B_1, $\mu g/kg$*
	25 °C		
1.0	12	14	48
1.0	16	18	27
1.5	16	18-37	15†
2.0	17	18	15‡
4.0	22.5	18	6
	49 °C		
0.3	12	14	63
1.0	16	6	3§
1.5	16	3	4
		6	1
2.0	17	6	2

*Initial aflatoxin B_1 content = 260 $\mu g/kg$.
†Average of five experiments. Rel. std. dev. = 37 percent.
‡Average of two experiments. Rel. std. dev. = 28 percent.
§Average of three experiments. Rel. std. dev. = 45 percent.

15 $\mu g/kg$. Extension of the reaction period would have been helpful and, based upon the data in Fig. 1, a 14-day reaction period at 25 °C presumably would have reduced the residual B_1 level by possibly 30 percent to about 10 $\mu g/kg$.

The 18 samples (2 replications in Table 2) mentioned above as having an average residual B_1 level of 8.6 $\mu g/kg$ had a RSD of 23 percent. This value is considered satisfactory in view of a RSD of 25 percent reported by Shotwell et al. (1975a) for duplicate aflatoxin determinations made on samples of naturally contaminated corn.

Ammonia Diffusion and Addition

When the ammonia had a chance to diffuse, uniform application of ammonia to all parts of the corn was not necessary. For instance, when 22.7 kg of untreated corn (lot 108, 450 μg aflatoxin B_1/kg) were mixed with 22.7 kg of this corn ammoniated at the 1 percent level 1 day earlier, the residual aflatoxin B_1 content after 14 days at 25 °C was 34 $\mu g/kg$. Treated corn moisture was 12.6 percent. This result agreed with the 28 $\mu g/kg$ of aflatoxin B_1 in a sample of the corn treated with 0.5 percent ammonia at 14.5 percent moisture and held 14 days at 25 °C.

Because side reactions of both known and unknown nature can occur between various corn components and ammonia and thus reduce the availability of ammonia for aflatoxin inactivation, the question arose whether or not addition periodically of a given quantity of ammonia in increments to provide a fresh supply would be more effective than a single addition. With the same starting and completion dates for detoxification, incremental addition of ammonia at intervals of several days was neither more nor less effective than addition of the full amount initially. Because a 1 percent addition of ammonia gave an ammonia:aflatoxin B_1 ratio on a molal basis of over 1.8 x 10^5 to 1 for the corn lots used, incremental addition of the ammonia would not alter the mass action effect for detoxification but could do so for possible side reactions. The latter possibility was not investigated, however.

The maximum amount of ammonia that can be added without surface runoff in one pass of corn through a screw conveyor is about 1 percent, based on an aqua ammonia concentration of 25 weight percent. Thus, addition of greater amounts of ammonia would require multiple additions with a holding period for absorption of the liquid before more is added. Because all the ammonia need not be added the same day for effective detoxification, the operator thus has more flexibility in his operation.

Response of Various Corn Lots to Ammoniation

Initial aflatoxin level affects the degree of inactivation obtained. Samples from 8 lots of corn, varying in aflatoxin B_1 content from 30 to 1000 $\mu g/kg$, were treated with 1 percent ammonia and held at 25 °C for 14 days after adjustment of the corn moisture to 14-15 percent. For initial aflatoxin B_1 levels up to 140 $\mu g/kg$ this treatment resulted in ND levels of B_1 (Table 3). Lot 103 (160 $\mu g/kg$) may well have been reduced to a ND level also if the moisture content had been adjusted to 15 percent. At 440-450 $\mu g/kg$, the treatment reduced the B_1 level to 9 $\mu g/kg$, and for 1000 $\mu g/kg$ initially, the residual B_1 level was 22 $\mu g/kg$ (extrapolated). These findings are in line with the behavior expected from the data shown in Fig. 1, assuming the reaction kinetics do not change with initial aflatoxin level. That is to say that the shape of the curves for residual aflatoxin content vs reaction time is not dependent upon the initial aflatoxin level.

Another lot (104) with an initial aflatoxin content of 260 $\mu g/kg$ was particularly difficult to detoxify, for reasons we have been unable to discover. By interpolation, the ammonia treatment used on the 8 lots listed in Table 3 (i.e., 1 percent ammonia, 15 percent corn moisture, 25 °C for 14 days) would have reduced the aflatoxin B_1 content of lot 104 to about 35 $\mu g/kg$ (Table 4). Increasing the ammonia level, corn moisture level and reaction time at 25 °C lowered the residual B_1 level of lot 104 to 6-15 $\mu g/kg$. Elevation of the reaction temperature to 49 °C made the ammoniation treatment more effective and further reduced the aflatoxin B_1 content to levels of 1-4 $\mu g/kg$, but in no case was the residual aflatoxin B_1 reduced to a ND level under the conditions used. Lot 104, however, should not be considered a typical corn because it had been grown on irrigated land in a southwestern state. In other respects it was an excellent corn and graded U.S. No. 1.

TABLE 5. TOXICITY FEEDING TESTS ON AMMONIATED CORN*.

Test animal	Aflatoxin B_1 reduction by ammoniation µg/kg	Length of feeding period	Aflatoxin response	Residual $WE\text{-}NH_3$†, percent d.b.	Ammoniation temperature, °C	Reaction time
Duckling	230→ND	4 days	ND	0.27	18-38	ca. 7 mos.
Broiler chick	1200→16	3 weeks	ND	0.17	25-47	19 days
Trout	160→ND	12 mos.	ND§	0.15	49	13 days

*After 1.0 percent NH_3 had been added and corn moisture adjusted to 17.5 percent (w.b.), the corn was held for indicated time and temperature, then ground, heated, and aerated to remove the ammonia odor.
†Water-extracted ammonia in ground, deodorized corn.
‡Non detected.
§See text (section entitled "Animal Acceptance of Ammoniated Corn") for further information.

Biological Confirmation of Chemical Assays for Aflatoxin Content

While chemical assays were helpful in monitoring the ammoniation experiments, the assays could be misleading if not substantiated by biological tests. Fortunately, biological assays made with ducklings, broiler chicks, and trout did substantiate the chemical assays. Although the duckling test is only a semi-quantitative assay (Legator, 1969), it is widely used because the test can be completed in a few days, and the duckling is very sensitive to aflatoxin. In this instance, the ammoniated corn was incorporated directly into the duckling diet rather than relying upon intubation of an extract into the digestive tract, because the extract might not adequately reflect the level and nature of any residual aflatoxin or any toxic products present in the ammoniated corn. Aflatoxin response of the ducklings to our ammoniated corn sample having a chemically nondetectable aflatoxin content was essentially negative.

Broiler chicks were fed for 21 days on a ration containing good corn, or aflatoxin-contaminated corn, or the ammoniated counterpart of either. The data obtained on internal organ weights, serum analyses, and body weight gain indicated that ammoniation, which had reduced aflatoxin B_1 content of the corn from 1200 to 16 µg/kg based on chemical assays, did eliminate the acute toxicity of the aflatoxin (Hamilton et al., 1977).

Rainbow trout are very sensitive to aflatoxin and during a 12-month feeding test reportedly can detect the toxin at a level as low as 1 µg/kg in the ration. After such a test, a slight indication of small tumor development was found in the livers of a few trout fed a ration containing the ammoniated, aflatoxin-decontaminated corn, good corn or the basal ration (Brekke et al. 1977). In his report on the trout test, Sinnhuber (1974) stated that "ammoniation of corn by your process was effective in destroying aflatoxin B_1 but perhaps other substances might be present which could be the cause of the slightly unusual histology." The ammoniation process did not diminish the nutritive value of the corn to rainbow trout.

Conditions used in ammoniating the corn used for each of these tests, the degree of aflatoxin reduction obtained for each, and other data are summarized in Table 5.

Animal Acceptance of Ammoniated Corn

Ammoniated corn can have a very strong ammonia odor which diminishes slowly with time in a closed container or more rapidly upon exposure to air and heat. After ducklings refused to eat the undeodorized sample, subsequent samples for the biological tests were ground, heated, and aerated to remove essentially all ammonia odor. After deodorization, these ground samples analyzed 0.15-0.27 percent $WE\text{-}NH_3$ (d.b.) (Table 5).

To learn more about the ammonia tolerance level, two collaborative feeding tests of a short-term nature were made wherein corn ammoniated at several levels and not deodorized was fed to laying hens and swine. In feeding tests conducted over a 5-wk period by C. C. Calvert (1976), laying hens readily ate a ground, mixed, standard laying hen ration containing corn ammoniated at the 0.5 or 1.0 percent level. There was no reduction in egg production but because of their age, the hens' egg production rate had passed its peak. Feed consumption fell off some for hens fed the diet containing 1.5 percent ammoniated corn and still further when the corn had been treated with 2.6 percent ammonia. Fresh feed was placed before the hens every second day. They backed off from the freshly placed feed but ate it after a few hours. Very little ammonia odor was noted when rations for the two lower ammoniation levels were mixed but those prepared with the 1.5 and 2.6 percent ammoniated corn had a pronounced odor. Samples of ground rations, analyzed 20 wks after the corn had been ammoniated had no detectable level of $WE\text{-}NH_3$ for the three lowest ammoniation levels; the sample containing corn treated with 2.6 percent ammonia analyzed 0.24 percent $WE\text{-}NH_3$. The whole corn samples contained 0.10, 0.29, 0.46, and 0.86 percent $WE\text{-}NH_3$ for the respective ammoniation levels 20 wks after ammoniation. (See next section on Ammonia Accountability re differences in $WE\text{-}NH_3$ values between whole and ground corn.) In a taste-panel test, eggs collected during the feeding trial reportedly had no off flavor irrespective of the ammoniation level (Dizikes, 1976). The eggs were tasted as both scrambled and hardboiled and, in the latter case, the whites and yolks were tasted separately.

In an acceptance test limited to a few days duration, Jensen et al. (1977) fed ammoniated corn along with a high protein (i.e., soybean meal)-mineral-vitamin supplement to swine of about 63 kg average weight. The ingredients were ground and mixed so the swine could not selectively exclude any particular ingredient. The mixed ration containing corn treated with 0.5 percent ammonia was readily consumed. Feed consumption at the higher ammoniation levels declined and

TABLE 6. DRYING TEST ON AMMONIATED CORN TO REDUCE AMMONIA ODOR AND TASTE*.

Drying time, hr	M&VM, percent, w.b.	$WE\text{-}NH_3$, percent, d.b.†	NH_3 odor‡	Burning taste §
0	18.5	0.50	VS	S
0.2	16.1	0.42	S	S
0.8	14.9	0.38	S	S
1.5	13.1	0.36	VS	M
2.5	11.7	0.34	S	Sl
3.5	10.9	0.33	S	Sl
4.2	10.3	0.32	M	Sl
6.9	10.6	0.31	S	Sl
9.5	10.1	0.29	M	Mom
22.5	8.5	0.27	M	Tr?

*Corn treated with 1 percent NH_3 was held 23 days at 24-32 °C before drying. Layer of corn 2.5 cm deep was dried with 51 °C air in recirculating (downflow) laboratory tray dryer. Samples were held 40 hr in closed containers at room temperature before odor and taste tests were made.

†In whole corn.

‡VS = very strong, S = strong, M = moderate.

§S = strong, M = moderate, Sl = slight, Mom = momentary, Tr = trace, or slightly burnt taste because of drying. Taste test made by one individual chewing 10 kernels.

at the 2 percent ammonia level the consumption level fell to about half that of the 0.5 percent level. When offered free choice (i.e., the hog could freely eat whatever amount he desired of whole corn and supplement), consumption of corn to which 0.5 percent ammonia had been added was less than half that of nonammoniated corn. Consumption decreased further as 1.0, 1.5, or 2.0 percent ammonia was added to the corn.

Drying Treated Corn Reduces Ammonia Odor and Taste

The duckling test and animal acceptability studies of Drs. Jensen and Calvert suggested that drying to increase acceptability of the ammoniated corn was necessary. The results of one drying test and the qualitative, subjective evaluations made of samples taken during the test are given in Table 6. Plots of total volatiles and $WE\text{-}NH_3$ against time gave similar, typical drying curves. While the data on hand from several drying tests did not provide a completely definitive answer, drying ammoniated corn to under 12 percent moisture and volatile material (M&VM) removed quite a bit of the $WE\text{-}NH_3$. When the dried corn is ground and mixed with other ingredients as in a mixed feed, residual ammonia content presumably would not be objectionable. Present indications are that drying the ammoniated corn to 9 to 11 percent M&VM will prove satisfactory as was done in preparation of corn for additional swine and poultry feeding tests.

Ammonia Accountability

Based upon their content of ammoniacal nitrogen, total ammonia content of corn samples held 14 days at 25 °C and then neutralized with acid closely approximated the amount of ammonia added in carefully conducted experiments (Fig. 3). All values shown in Fig. 3 are the average of four determinations made on corn samples adjusted to moisture contents of 12.5, 15.0, 17.5, and 20.0 percent during treatment with the specified amount of ammonia. Only part of the added ammonia was water extractable, the remainder being considered fixed as stated by Lancaster et al. (1974). More $WE\text{-}NH_3$ was recovered from the ground samples (through No. 6 U.S. sieve with 2.38 mm openings) than from whole corn samples at the 0.5 percent ammonia-

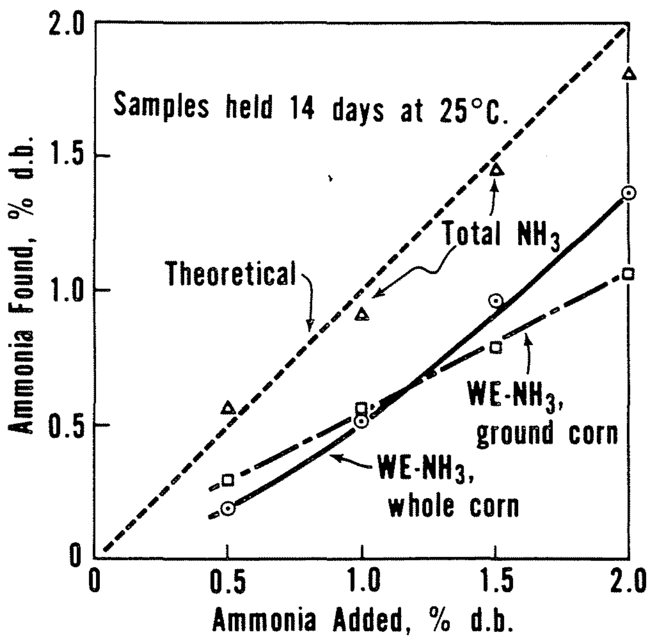

FIG. 3 Correlation between ammonia added and levels of total ammonia and water-extracted ammonia [$WE\text{-}NH_3$] found in corn after samples were held 14 days at 25 °C.

tion level and less at the 1.5 and 2.0 percent levels. These differences are attributed to a relatively high proportion of fixed ammonia in corn treated with 0.5 percent ammonia, and to the loss of ammonia upon grinding unneutralized samples treated with 1.5 or 2.0 percent ammonia. In this series of experiments, the $WE\text{-}NH_3$ values for determinations made on whole corn were 36, 52, 65, and 69 percent of the ammonia added for the 0.5, 1.0, 1.5, and 2.0 percent addition levels, respectively. These and other unreported data indicate that at ammonia addition levels of 1.5 percent and above, the $WE\text{-}NH_3$ values level off at about two-thirds of the ammonia added.

Some Physical Characteristics of Ammoniated Corn

One readily observable characteristic of ammoniated corn is the change in color. White corn develops a yellowish or tannish color whereas yellow corn can develop a color ranging from slight discoloration to a dark tan and even a deep mahogany color depending upon reaction conditions, such as ammonia addition level, corn moisture, reaction temperature, and time. The ammoniated pigments are largely water soluble because much of the orignial color returns upon soaking the corn in water for the $WE\text{-}NH_3$ determination.

Another and particularly annoying characteristic from the material-handling standpoint is a tendency for the corn to cake due to surface stickiness that develops after ammonia is applied. The stickiness appears to be more pronounced at higher moisture levels, which suggests that moisture accentuates the ammonia-corn component reaction causing the stickiness. Nature of the reaction involved is unknown.

Safety Considerations

Certain safety precautions are necessary in handling ammoniated corn. Adequate ventilation is recommended to avoid exposure of workmen to vapor concentrations above the maximum specified by safety authorities.

Exposure to ammonia vapors can lead to headaches, diarrhea, nausea, and temporary weakness. Handlers and users of aqua ammonia are or should be familiar with other recommended safety practices which need not be reviewed here.

While the effect upon man has not been fully established, aflatoxin is a potential carcinogen. Therefore, in handling contaminated grain, certain safety precautions should be followed. Dust respirators should be used, and the head and skin (especially breaks due to cuts, etc.), protected from direct contact with contaminated dust and grain by use of protective clothing, preferably of the disposable type. For decontamination after exposure, the recommended practice is to wash the hands and forearms with household bleach (5 to 6 percent sodium hypochlorite, i.e. Clorox), preferably full strength, otherwise diluted one-half, and then with soap and water. If the skin is too sensitive to sodium hypochlorite solution, sodium perborate with a detergent may be used. Disposable clothing and gloves should be burned. Contaminated garments which are to be laundered should first be soaked for an hour in 5 percent sodium carbonate solution or in 1 percent bleach solution depending upon type of fabric and coloring (Goldblatt, 1969b).

Safety procedures developed in handling and ammoniating large quantities of contaminated corn are given in another report (Brekke et al., 1976).

SUMMARY AND CONCLUSIONS

In laboratory tests, aqua ammonia effectively eliminated the acute toxicity of aflatoxin in naturally contaminated whole corn. Chemical assays of neutralized samples were used to follow the course of inactivation. Biological feeding tests made with ducklings, broiler chicks, and trout on certain samples of ammoniated corn established validity of chemical assays.

The inactivation reaction was highly temperature sensitive over the range of - 18 to 60 °C and became more effective as the corn moisture was increased from 12 1/2 to 17 1/2-20 percent. As the aflatoxin inactivation proceeded, further inactivation required progressively more time. With 1.5 percent ammonia added to corn, and the latter's moisture content adjusted to 17.5 percent, the aflatoxin B_1 content of one lot was reduced from 1000 to under 20 μg/kg (i.e., 20 ppb; in 42 days at 10 °C, or 8 days at 25 °C, or 2 days at 40 °C. Use of more ammonia, i.e., 2 to 4 percent, generally proved to be of little additional benefit; lesser amounts, i.e., 0.5 and 1 percent, reduced the aflatoxin content considerably but the reduction was not always sufficient to meet the Food and Drug Administration (FDA) guideline. FDA currently specifies 20 ppb total aflatoxin content as action guideline in feed ingredients shipped in interstate commerce. Several aflatoxins may be present in corn and usually more than 75 percent of the total is the B_1 type. Use of ammoniated corn presumably will be limited to feeding animals.

Degree of aflatoxin inactivation differed among various lots of corn depending upon initial aflatoxin content and in some instances upon other unknown factors. Reduction of residual aflatoxin content to a nondetectable level became more difficult as initial aflatoxin level increased.

Ammoniated corn generally had a strong odor and burning taste due to the ammonia, and the intensity increased with ammoniation level. Acceptance by swine and poultry of the treated grain (not deodorized) decreased as ammonia addition level increased. When fed a ground, mixed feed, swine readily ate a ration containing corn treated with 0.5 percent ammonia. Laying hens also readily ate ground, mixed rations incorporating corn ammoniated at the 0.5 percent and 1.0 percent levels. Feed consumption fell off at the higher ammoniation levels for both animal species.

Laboratory tests on whole corn containing 18-19 percent moisture and treated with 0.5 or 2.0 percent ammonia demonstrated that drying the corn to 9-11 percent volatiles content eliminates or reduces the odor and burning taste and thus increases animal acceptance.

Before the USDA can recommend a detoxification process for corn, approval by the FDA is needed of the ammoniated corn and indirectly of the ammoniation process. Feeding tests to obtain such approval were started in late 1975 with swine and laying hens on corn ammoniated by a recycle gas-phase method (Brekke et al., 1976) with process conditions based in part upon information developed in the present study. (Details on the feeding results will be described in subsequent papers.) Excessive loss of ammonia during transfer by bucket elevator and spouting of corn freshly wetted with aqua ammonia has delayed development of a practical process using the aqua form.

Ammoniation darkens the corn and causes surface stickiness which leads to a material handling problem because of caking in a static bed.

Work on several aspects of the program is continuing or is being planned.

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